Eur. J. Clin. Chem. Clin. Biochem.
Vol. 31, 1993, pp. 121–128

Biochemical Diagnosis of a Fatal Case of *Günther*'s Disease in a Newborn with Hydrops Foetalis

By *L. Verstraeten*[1], *N. Van Regemorter*[2], *A. Pardou*[3], *H. de Verneuil*[4], *V. Da Silva*[6], *F. Rodesch*[5], *D. Vermeylen*[3], *C. Donner*[2], *J. C. Noël*[2], *Y. Nordmann*[6] and *A. Hassoun*[1]

[1] *Department of Clinical Biochemistry, Université Catholique de Louvain, Cliniques Universitaires St Luc, Bruxelles, Belgique*
[2] *Department of Genetics, Faculté de Médecine, Université Libre de Bruxelles, Bruxelles, Belgique*
[3] *Department of Neonatal Intensive and Non-Intensive Care Unit, Hôpital Universitaire Erasme, Université Libre de Bruxelles, Bruxelles, Belgique*
[4] *Department of Clinical Biochemistry, Université de Bordeaux II, Bordeaux, France*
[5] *Department of Gynecology and Obstetrics, Hôpital Universitaire Erasme, Université Libre de Bruxelles, Bruxelles, Belgique*
[6] *Department of Biochemistry, Faculté de Médecine Xavier Bichat, Hôpital Louis Mourier, Colombes, France*

(Received September 23/November 20, 1992)

Summary: The birth of a male baby was induced at 32 weeks. In utero, the child presented, inter alia, signs of hydrops, hepatosplenomegaly and anaemia. Two in utero transfusions for correction of the anaemia were performed at 28 and 29 weeks, respectively. The baby rapidly presented respiratory distress with mixed acidosis. Three hours after birth, pink urine was excreted. Signs of icterus necessitated phototherapy, after which photosensitivity occurred. Erythrocytes were fluorescent under long-wavelength UV light. The baby died 24 hours after birth, displaying severe acidosis, a diffuse haemorrhagic syndrome, and repeated bradycardia which did not respond to isoprenaline.

The analysis of porphyrins in urine, blood and faeces of the baby gave the following results:

1) uroporphyrin (I and III isomeric series) was increased in urine and faeces, with traces in erythrocytes and plasma;

2) heptacarboxyporphyrin I was found mainly in urine and much less in erythrocytes, plasma and faeces;

3) coproporphyrin I was increased in urine, erythrocytes, plasma and faeces, and

4) 5-aminolaevulinic acid and porphobilinogen in urine and plasma were within the reference ranges.

Determination of the enzymes of haem biosynthesis in erythrocytes and lymphocytes showed that both parents possessed only 50% of the normal activity of cosynthase.

A previously described point mutation in codon 73 was observed in one parent. Fatal cases of neonatal *Günther*'s disease are extremely rare and such an observation, according to our knowledge, is probably one of the first described.

Introduction

Günther's disease, also called congenital erythropoietic porphyria (CEP), is a very rare (less than 200 cases reported) type of porphyria (1, 2). Congenital erythropoietic porphyria is characterized by an overproduction of porphyrins of isomer I series, with accumulation of these compounds in bone marrow erythroblasts, teeth and bones (1, 2). These porphyrins

are excreted in excess in urine and faeces (1, 2). Patients with congenital erythropoietic porphyria show an inability to maintain the normal production of porphyrins of isomer III type, consistent with diminished activity of uroporphyrinogen III synthase[1]) (or cosynthetase or hydroxymethylbilane hydrolase cyclizing, EC 3.2.1.75) (3). Congenital erythropoietic porphyria is inherited as a *Mendel*ian autosomal recessive trait (4). The onset of the majority of cases occurs before the 6th year of life and the main symptoms are cutaneous photosensitivity and haemolysis (5). Congenital erythropoietic porphyria is very rarely detected in utero or at birth (6, 7, 8).

Our patient is a male premature neonate. Birth was induced at 32 weeks, because he presented in utero, inter alia, signs of hydrops, hepatosplenomegaly and anaemia. Two in utero transfusions for correction of the anaemia were performed at 28 and 29 weeks, respectively. The patient rapidly presented respiratory distress with mixed acidosis.

Three hours after birth, pink urine was excreted. Signs of icterus necessitated phototherapy, after which marked photosensitivity occurred. Red blood cells and bone marrow erythroblasts were fluorescent (red) under long-wavelength UV light.

The baby died 24 h after birth, displaying severe acidosis, a diffuse haemorrhagic syndrome, and repeated bradycardia which did not respond to isoprenaline (Isuprel®).

This study describes the contribution of biochemical investigations to the diagnosis of this rare type of porphyria, associated with unusual clinical onset. We also report the investigation of porphyrin metabolism in the parents.

[1]) 5-Aminolaevulinic acid dehydratase (EC 4.2.1.24)
Porphobilinogen deaminase (EC 4.2.1.75)
Uroporphyrinogen III cosynthase (EC 4.3.1.8)
Uroporphyrinogen decarboxylase (EC 4.1.1.37)
Coproporphyrinogen oxidase (EC 1.3.3.3)
Protoporphyrinogen oxidase (EC 1.3.3.4)

Materials and Methods

Urinary 5-aminolaevulinic acid and porphobilinogen were determined spectrophotometrically after elution from ion-exchange resins (Bio-Rad, Germany), according to *Mauzerall & Granick* (9).

Tab. 1. Erythrocyte porphyrins

Porphyrins		Patient	Father	Mother	Reference values
Uroporphyrin fraction	(nmol/l)	traces	32	26	< 50
Octacarboxyporphyrin	(nmol/l)	traces	32	26	
Heptacarboxyporphyrin	(nmol/l)	traces	ND	ND	
Coproporphyrin fraction	(nmol/l)	1715	34	71	<100
Hexacarboxyporphyrin	(nmol/l)	ND	7	10	
Pentacarboxyporphyrin	(nmol/l)	ND	9	8	
Tetracarboxyporphyrin	(nmol/l)	1715	18	53	
Protoporphyrin fraction	(nmol/l)	58	110	163	<500
Protoporphyrin	(nmol/l)	48	43	141	
Zinc protoporphyrin	(nmol/l)	10	67	22	
Isomer fractionation					
Octacarboxyporphyrin					
Isomer I	(%)	33	40	60	< 30
Isomer III	(%)	67	60	40	> 70
Heptacarboxyporphyrin					
Isomer I	(%)	100	ND	ND	< 30
Isomer III	(%)	ND	ND	ND	> 70
Hexacarboxyporphyrin					
Isomer I	(%)	ND	100	100	< 30
Isomer III	(%)	ND	ND	ND	> 70
Pentacarboxyporphyrin					
Isomer I	(%)	ND	45	ND	< 30
Isomer III	(%)	ND	55	100	> 70
Tetracarboxyporphyrin					
Isomer I	(%)	95	40	27	< 30
Isomer III	(%)	5	60	73	> 70

Urinary and faecal porphyrins were determined by high performance liquid chromatography (HPLC) as previously described (9).

Plasma porphyrins were determined by HPLC, using a method derived from that of *Longas & Poh-Fitzpatrick* (10).

Erythrocyte porphyrins were determined by HPLC, using a method derived from that of *Scoble* et al. (11).

Isomers I and III in urine, faeces, plasma and erythrocytes were fractionated by HPLC, using a method derived from that of *Lim* et al. (12).

Uroporphyrinogen III synthase activity in erythrocytes (both parents) was determined as previously described (8).

5-Aminolaevulinate dehydratase (EC 4.2.1.24), porphobilinogen deaminase (EC 4.3.1.8) and uroporphyrinogen decarboxylase (EC 4.1.1.37) activities[1]) were determined in erythrocytes as previously described (9, 13).

Coproporphyrinogen oxidase (EC 1.3.3.3) and protoporphyrinogen oxidase (EC 1.3.3.4) activities[1]) were determined in lymphocytes as previously described (14, 15).

Results

Some features of the clinical investigation (emission of pink urine, hirsutism, photosensitivity) and the erythrocyte fluorescence under long-wavelength UV led us to a more detailed investigation of porphyrin metabolism, including the fractionation of isomers I and III, and determination of the enzymes of haem biosynthesis. This study was performed on the patient and his two parents.

Tables 1 to 4 present the results of porphyrin determinations in plasma, erythrocytes, urine and faeces.

In the patient, tetracarboxyporphyrin (almost exclusively isomer I) was strongly increased in erythrocytes, plasma, urine and faeces. Octacarboxyporphyrin (a mixture of isomers I and III) was strongly increased in urine and to a lesser degree in faeces. Heptacarboxyporphyrin (isomer I) was increased in urine, and to a lesser degree in plasma, erythrocytes and faeces.

Table 5 shows the results for several enzymes of haem biosynthesis.

Discussion

Although one of the rarest of the porphyrias, congenital erythropoietic porphyria was the first of these diseases recorded in the literature, probably because

Tab. 2. Plasma porphyrins and precursors

		Patient	Father	Mother	Reference values
5-Aminolaevulinic acid (μmol/l)		not determined	0.1	0.1	< 0.6
Porphobilinogen (μmol/l)		not determined	0.1	0.2	< 0.2
Uroporphyrin fraction	(nmol/l)	6	traces	traces	< 5
Octacarboxyporphyrin	(nmol/l)	traces	traces	traces	
Heptacarboxyporphyrin	(nmol/l)	6	ND	ND	
Coproporphyrin fraction	(nmol/l)	4053	4	12	<10
Hexacarboxyporphyrin	(nmol/l)	ND	ND	ND	
Pentacarboxyporphyrin	(nmol/l)	ND	4	12	
Tetracarboxyporphyrin	(nmol/l)	4053	ND	traces	
Protoporphyrin (nmol/l)		54	27	41	<50
Isomer fractionation					
Octacarboxyporphyrin					
Isomer I	(%)	50	ND	50	<30
Isomer III	(%)	50	100	50	>70
Heptacarboxyporphyrin					
Isomer I	(%)	100	ND	ND	<30
Isomer III	(%)	ND	ND	ND	>70
Hexacarboxyporphyrin					
Isomer I	(%)	ND	ND	ND	<30
Isomer III	(%)	ND	ND	ND	>70
Pentacarboxyporphyrin					
Isomer I	(%)	ND	ND	35	<30
Isomer III	(%)	ND	100	65	>70
Tetracarboxyporphyrin					
Isomer I	(%)	100	ND	100	<30
Isomer III	(%)	ND	ND	ND	>70

Tab. 3. Urine porphyrins and precursors

	Patient	Father	Mother	Reference values
5-Aminolaevulinic acid (μmol/g creatinine)	26	8	10	< 25
Porphobilinogen (μmol/g creatinine)	ND	1	1	< 5
Uroporphyrin fraction (nmol/g creatinine)	166539	31	10	<110
Octacarboxyporphyrin (nmol/g creatinine)	154920	31	10	
Heptacarboxyporphyrin (nmol/g creatinine)	11619	ND	traces	
Coproporphyrin fraction (nmol/g creatinine)	266232	340	386	<740
Hexacarboxyporphyrin (nmol/g creatinine)	ND	ND	ND	
Pentacarboxyporphyrin (nmol/g creatinine)	18682	ND	ND	
Tetracarboxyporphyrin (nmol/g creatinine)	247549	340	386	
Isomer fractionation				
Octacarboxyporphyrin				
Isomer I (%)	43	50	35	< 30
Isomer III (%)	57	50	65	> 70
Heptacarboxyporphyrin				
Isomer I (%)	100	ND	60	< 30
Isomer III (%)	ND	ND	40	> 70
Hexacarboxyporphyrin				
Isomer I (%)	ND	ND	ND	< 30
Isomer III (%)	ND	ND	ND	> 70
Pentacarboxyporphyrin				
Isomer I (%)	100	ND	100	< 30
Isomer III (%)	ND	ND	ND	> 70
Tetracarboxyporphyrin				
Isomer I (%)	99	97	45	< 30
Isomer III (%)	1	3	55	> 70

of the severity of its cutaneous symptoms (16). Congenital erythropoietic porphyria is a very rare disease (less than 200 cases reported in the literature). Higher prevalences reported previously are explained by confusion with early-onset cases of cutaneous hepatic porphyria (17, 18, 19).

The pattern of porphyrin overproduction in congenital erythropoietic porphyria indicates that the block in the haem synthetic pathway occurs at the level of uroporphyrinogen III synthase (3, 20–25). This enzyme catalyses the formation of uroporphyrinogen III from hydroxymethylbilane or from porphobilinogen if porphobilinogen deaminase is present (2). Patients with congenital erythropoietic porphyria demonstrate an inability to maintain the normal production of series III isomers, consistent with a diminished activity of uroporphyrinogen III synthase (3).

Erythrocyte uroporphyrinogen III synthase activity in both parents of our patient is about 50% of the normal value, which is compatible with an heterozygous state for congenital erythropoietic porphyria (26).

Although there is a deficiency of uroporphyrinogen III synthase, it does not completely block the synthesis of series III isomer porphyrins (1, 2). Indeed, as stated previously, there is some increase in total porphyrin synthesis, as evidenced by increased concentrations in peripheral blood and urine (1, 2). In our patient, we observed a large predominance of type-I isomers of tetra; penta- and heptacarboxyporphyrins in all the biological material examined (erythrocytes, plasma, urine and faeces). The octacarboxyporphyrins were detected in small amounts in erythrocytes, plasma and faeces, and high quantities were excreted in urine.

Tab. 4. Faccal porphyrins

Porphyrins	Patient	Father	Mother	Reference values
Uroporphyrin fraction (nmol/g dry weight)	22	3	6	ND
Octacarboxyporphyrin (nmol/g dry weight)	14	1	4	
Heptacarboxyporphyrin (nmol/g dry weight)	8	2	2	
Coproporphyrin fraction (nmol/g dry weight)	2132	34	10	< 110
Hexacarboxyporphyrin (nmol/g dry weight)	ND	ND	ND	
Pentacarboxyporphyrin (nmol/g dry weight)	43	ND	ND	
Tetracarboxyporphyrin (nmol/g dry weight)	2089	34	10	
Protoporphyrin (nmol/g dry weight)	61	1	1	< 90
Isomer fractionation				
Octacarboxyporphyrin				
Isomer I (%)	35	65	40	< 30
Isomer III (%)	65	35	60	> 70
Heptacarboxyporphyrin				
Isomer I (%)	100	ND	20	< 30
Isomer III (%)	ND	100	80	> 70
Hexacarboxyporphyrin				
Isomer I (%)	ND	ND	ND	< 30
Isomer III (%)	ND	ND	ND	> 70
Pentacarboxyporphyrin				
Isomer I (%)	100	ND	ND	< 30
Isomer III (%)	ND	ND	ND	> 70
Tetracarboxyporphyrin				
Isomer I (%)	100	85	80	50–70
Isomer III (%)	ND	15	20	30–50

Tab. 5. Haem biosynthesis enzymes determination

Enzyme	Father	Mother	Reference values
Erythrocyte 5-aminolaevulinate dehydratase (5-aminolaevulinate, µmol/min · l erythrocytes at 37 °C)	38	43	20 – 60
Erythrocyte porphobilinogen deaminase (uroporphyrin, pmol/h · mg haemoglobin at 37 °C)	142	178	103 – 243
Erythrocyte uroporphyrinogen cosynthase (cosynthase, units/mg protein at 37 °C)	5.0	5.5	8.0 – 12.0
Erythrocyte uroporphyrinogen decarboxylase (coproporphyrinogen, nmol/h · mg protein at 37 °C)	40	36	30 – 70
Lymphocyte coproporphyrinogen oxidase (protoporphyrin, pmol/mg protein at 37 °C)	706	499	350 – 650
Lymphocyte protoporphyrinogen oxidase (protoporphyrin, nmol/h · mg protein at 37 °C)	5.2	5.0	3.6 – 6.0

In all these biological fluids, the ratio of isomer I to isomer III varied from 30 to 50%.

Furthermore, increased concentrations of erythrocyte porphyrins have been found in relatives of congenital erythropoietic porphyria patients, consistent with a partial deficiency of uroporphyrinogen III synthase in heterozygotes (3, 27–29). In our study, plasma and erythrocyte porphyrins were within reference range for both parents. The patient showed extremely high concentrations of plasma and erythrocyte coproporphyrin, almost exclusively of the type-I isomer.

Typically, the urinary total porphyrin concentration in congenital erythropoietic porphyria is between 20 000 and 90 000 nmol/l (2). This consists mainly of uroporphyrin and heptacarboxylic porphyrin, with 10–20% coproporphyrin (30, 31). In our patient, urine total porphyrin concentration was about 430 000 nmol/l. Coproporphyrin (almost exclusively type-I isomer) is predominant in urine. Both parents have urine porphyrin values within the reference range, but the father excretes predominantly type-I coproporphyrin. It is to be noted that the coproporphyrin in normal urine consists of 70% isomer III and 30% isomer I (32). An increased ratio of coproporphyrin I to coproporphyrin III may be observed in other diseases such as cholestasis and *Dubin-Johnson* syndrome (33, 34). Excessive production of intermediate porphyrins, as found by HPLC, also occurs in cutaneous hepatic porphyria, but the pattern of excreted compounds is quite different (1, 2). Isomeric fractionation shows that more than 80% of the total porphyrin is of series I, although, as stated before, there is also an overall increase in the production and excretion of uroporphyrinogen III (1, 2). Concerning uroporphyrin, isomeric fractionation reveals a mixture of isomers I and III in all samples examined, whereas the typical pattern shows a predominance of type-I isomers (1). The predominance of uroporphyrin I over uroporphyrin III may also be observed in cases of cutaneous hepatic porphyria (35).

Faecal porphyrins are usually elevated in congenital erythropoietic porphyria, consisting predominantly of coproporphyrin, with a variable, but usually slight increase in protoporphyrin (1, 2). Uroporphyrin is also normally detectable in the faeces (1, 2). Unlike cutaneous hepatic porphyria, the isocoproporphyrin series of porphyrins is not detected (1, 2). In our patient, faecal coproporphyrin is strongly increased, together with a mild increase of uroporphyrin, hepta- and pentacarboxyporphyrins.

The strong increase of coproporphyrin in all biological samples led us to consider the diagnosis of a coexistent hereditary coproporphyria, an association previously described in a young female patient (36). This diagnosis was ruled out by the presence of normal values for lymphocyte coproporphyrinogen oxidase observed in both parents. The presence in all biological samples examined (erythrocytes, plasma, urine, faeces) of high concentrations of coproporphyrins, consisting of nearly 100% of the isomer I series, also excluded the diagnosis of hereditary coproporphyria in our patient (1, 2).

Concerning differential diagnosis, the laboratory investigations must be used to exclude other forms of porphyria, particularly any of the extremely rare cases of homozygous porphyria, as found in cutaneous hepatic porphyria and hereditary coproporphyria (1, 2). This diagnosis was excluded by the normal values of uroporphyrinogen decarboxylase and coproporphyrinogen oxidase in both parents, together with protoporphyrinogen oxidase.

With respect to the pathogenesis of cutaneous lesions (major photosensitivity observed after phototherapy, applied in this case because of icterus), many of the features of congenital erythropoietic porphyria differ only in severity from those found in other cutaneous porphyrias, such as cutaneous hepatic porphyria (1, 2). Our patient shows moderate hirsutism, which is commonly observed in cases of congenital erythropoietic porphyria (1, 2).

The majority of the patients presenting congenital erythropoietic porphyria suffer from haemolytic anaemia (1, 2). In this case, anaemia (haemoglobin concentration: 72 g/l) was diagnosed at 28 weeks and was treated by two in utero transfusions at 28 and 29 weeks. Indirect and direct *Coomb*'s tests were negative. Haemolysis seems to be associated with an increased osmotic fragility of erythrocytes, due to increased concentrations of erythrocyte porphyrins (37). Porphyrin deposition may also be found in bone marrow (38), where between 30 and 70% of the erythroblasts show porphyrin fluorescence (fluorocytes), i.e. bright red fluorescence is observed under long-wavelength UV light (38); this was also observed in our case.

The patient showed marked hepatosplenomegaly. In congenital erythropoietic porphyria, the liver in particular makes a significant contribution to excessive porphyrin synthesis (39). Splenomegaly is always observed in cases of congenital erythropoietic porphyria (1, 2). There are no characteristic histological findings in the spleen other than the manifestations of haemolysis (1, 2).

Congenital erythropoietic porphyria is expressed very rarely in utero, as is shown by the autopsy on a

newborn child (40), by diagnosis at birth (6) and by prenatal diagnosis (7, 8). Reddish-brown amniotic fluid with an increased porphyrin content is usually encountered early in the second trimester of pregnancy in fetuses with congenital erythropoietic porphyria (7, 41). In the present case, this fluid was colourless.

One of the earliest signs of the disease may be the excretion of reddish-coloured urine (1, 2). Emission of pink urine after three hours of life, together with a naso-pharyngal and gastric aspiration of pink liquid was observed in our case.

The patient shows the criteria of neonatal congenital erythropoietic porphyria, according to *Schwartz*:

1) emission of red urine together with an increase of uroporphyrins and coproporphyrins and normal concentrations of 5-aminolaevulinic acid and porphobilinogen;
2) presence of splenomegaly and haemolytic anaemia;
3) red fluorescence of the bone marrow (14).

Clinically, congenital erythropoietic porphyria may be distinguished from early-onset cutaneous hepatic porphyria by the presence of splenomegaly, erythrodontia (due to porphyrin accumulation in teeth, pathognomonic of congenital erythropoietic porphyria), anaemia and erythrocyte fluorescence (1, 2).

Congenital erythropoietic porphyria is inherited in an autosomal recessive fashion (1, 2). In this family, both parents are asymptomatic and heterozygous for congenital erythropoietic porphyria. Their first child was in good health. The second child died in utero at 33 weeks under very similar conditions to those observed in the present case (their third child): hydrops foetalis with hepatosplenomegaly compatible with an haemolytic anaemia (*Coomb*'s test was negative) and extramedullar erythropoiesis.

The isolation and sequencing of a full-length complementary DNA (cDNA) encoding uroporphyrinogen III synthase allows the study of the enzymatic defect at the molecular level (42). Different point mutations, insertions or deletions have been detected (43–45). It has now been shown that one of the parents carries the C 73 R mutation (a cysteine replaced by an arginine at position 73 in the protein), which has been previously described (43).

Conclusion

A fatal case of *Günther*'s disease (congenital erythropoietic porphyria) is reported in a baby whose birth was induced at 32 weeks. The biochemical diagnosis was made on the basis of:

1. a massive increase, in erythrocytes, plasma, urine and faeces, of type-I porphyrins (mainly coproporphyrin);
2. a familial study revealing that both parents are heterozygous for uroporphyrinogen III synthase deficiency;
3. the observation of a point mutation in codon 73 in one parent.

Fatal cases of neonatal congenital erythropoietic porphyria are extremely rare and such an observation, according to our knowledge, is probably one of the first described.

References

1. Moore, M. R., Mc Coll, K. E. L., Rimington, C. & Goldberg, A. (1987) Congenital porphyria. In: *Disorders of Porphyrin Metabolism*, pp. 213–226, Plenum Publishing Corporation, New York.
2. Kappas, A., Sassa, S., Galbraith, R. A. & Nordmann, Y. (1989) The porphyrias. In: *The Metabolic Basis of Inherited Diseases*, pp. 1305–1365 (Scriver, C. R., Beaudet, A. L., Sly, W. S. & Yale, D., eds.) Mac Graw-Hill Book Company, New York.
3. Romeo, G. & Levin, E. Y. (1969) Uroporphyrinogen III cosynthetase in human congenital erythropoietic porphyria. Proc. Natl. Acad. Sci. U. S. A. *63*, 856–869.
4. Darocha, T., Weremowicz, J. & Gregor, A. (1971) Family study in congenital erythropoietic porphyria. S. Afr. J. Lab. Clin. Med. *17*, 231–236.
5. Mascaro-Galey, C., Mascaro, J. M. & Albero, F. (1977) Porphyrie érythropoïétique congénitale de Günther chez une fillette de huit mois. Ann. Dermatol. Venerol. *104*, 32–37.
6. Martin, C., Babin, J. P., Sanjuan, B., Demarquez, J. L., Dubernet, C. & Maillet-Mezeray, G. (1973) Porphyrie érythropoïétique congénitale (maladie de Günther). Une observation diagnostiquée à la naissance. Nouv. Press. Med. *2*, 2591–2594.
7. Nitowsky, H. R., Sassa, S., Nakagawa, A. & Jagani, N. (1978) Prenatal diagnosis of congenital erythropoietic porphyria. Pediatr. Res. *12*, 455.
8. Deybach, J. C., Grandchamp, B., Grelier, M., Nordmann, Y., Boué, J., Boué, A. & de Béranger, P. (1980) Prenatal exclusion of congenital erythropoietic porphyria (Günther's disease) in a foetus at risk. Hum. Genet. *53*, 217–221.
9. Hassoun, A., Verstraeten, L., Mercelis, R. & Martin, J. J. (1989) Biochemical diagnosis of an hereditary aminolaevulinate dehydratase deficiency in a 63-year-old man. J. Clin. Chem. Clin. Biochem. *27*, 781–786.
10. Longas, M. O. & Poh-Fitzpatrick, M. B. (1980) High-pressure liquid chromatography of plasma free acid porphyrins. Anal. Biochem. *104*, 268–276.

11. Scoble, H. A., Mac Keag, M., Brown, P. R. & Kavaranos, G. J. (1981) The rapid determination of erythrocyte porphyrins using reverse-phase high performance liquid chromatography. Clin. Chim. Acta *113*, 253–265.
12. Lim, C. K., Ridcout, J. M. & Wright, D. J. (1983) Separation of porphyrin isomers by high performance liquid chromatography. Biochem. J. *211*, 435–438.
13. Elder, G. H. & Wyvill, P. C. (1982) Measurement of uroporphyrinogen decarboxylase using porphyrinogens prepared by chemical reduction. Enzyme *28*, 186–195.
14. Grandchamp, B. & Nordmann, Y. (1982) Coproporphyrinogen III oxidase assay. Enzyme *28*, 196–205.
15. Jacobs, N. J. & Jacobs, J. M. (1982) Assay for enzymatic protoporphyrinogen oxydation, a late step in heme biosynthesis. Enzyme *28*, 206–219.
16. Schultz, J. H. (1874) Ein Fall von Pemphigus leprosus, kompliziert durch Lepra visceralis. Inaugural Dissertation, Greifswald.
17. Gajdos, A., Gajdos-Török, M., Hartleyb, H. & Lausecker, C. (1963) Un cas de porphyrie congénitale traitée par l'acide adénosine 5 monophosphorique. Press. Med. *71*, 1294–1296.
18. Gajdos, A., Plainfosse, B. & Seringe, P. (1967) Porphyrie cutanée dite de l'adulte chez un enfant, révélée par une hépatite virale. Traitement par l'acide adénosine 5 monophosphorique. Press. Med. *75*, 545–547.
19. Schmid, R., Schwartz, S. & Sundberg, D. (1955) Erythropoietic congenital porphyria. Blood *10*, 416.
20. Levin, E. Y. (1975) Comparative aspects of porphyria in man and animals. Ann. NY. Acad. Sci. *244*, 481–492.
21. Watson, C. J., Runge, W., Taddeini, L., Bossenmaier, I. & Cardinal, R. (1964) A suggested control gene mechanism for the excessive production of types I and III porphyrins in congenital erythropoietic porphyria. Proc. Natl. Acad. Sci. USA. *52*, 478–485.
22. Eriksen, L. & Eriksen, N. (1977) Porphyrin distribution and porphyrin excretion in human congenital erythropoietic porphyria. Scand. J. Clin. Lab. Invest. *33*, 323–332.
23. Miyagi, K., Petryka, Z. J., Bossenmaier, I., Cardinal, R. & Watson, C. J. (1976) The activities of uroporphyrin synthesis and cosynthase in congenital erythropoietic porphyria. Am. J. Hematol. *1*, 3–21.
24. Moore, M. R., Thompson, G. G., Goldberg, A., Ippen, H., Seubert, A. & Seubert, S. (1978) The biosynthesis of haem in congenital erythropoietic porphyria. Int. J. Biochem. *9*, 933–938.
25. Mukerji, S. K., Pimstone, N. R., Gandhi, S. N. & Tan, K. T. (1985) Biochemical diagnosis and monitoring therapeutic modulation of disease activity in an unusual case of congenital erythropoietic porphyria. Clin. Chem. *31*, 1946–1951.
26. Deybach, J. C., de Verneuil, H., Phung, N., Nordmann, Y., Puissant, A. & Boffety, B. (1981) Congenital erythropoietic porphyria (Günther's disease), enzymatic studies on two cases of late onset. J. Lab. Clin. Med. *97*, 551–558.
27. Heilmeyer, L., Clotten, R., Kerp, L., Merker, H., Parra, C. A. & Wetzel, H. P. (1964) Porphyria erythropoietica congenita Günther – Ein Bericht über zwei Familien mit Erfassung der Merkmalsträger. Deutsche Med. Wochenschr. *88*, 2449–2456.
28. Heilmeyer, L. (1964) The erythropoietic porphyrias. Acta Haematol. (Basel) *31*, 137.
29. Pain, R. W., Welch, F. W., Woodroffe, A. J., Handley, D. A. & Lockwood, W. L. (1975) Erythropoietic uroporphyria of Günther first presenting at 58 years with positive family studies. Br. Med. J. *3*, 621–623.
30. Rimington, C. & With, T. K. (1973) Porphyrin studies in congenital erythropoietic porphyria. Studies on a sample of faeces and urine from a patient with CEP and comparison with a bovine case. Danish Med. Bull. *20*, 5–12.
31. Eriksen, L. & Eriksen, N. (1977) Urinary excretion of position isomers of penta and hexacarboxylated porphyrins belonging to the isomer III series in a case of human congenital erythropoietic porphyria. Scand. J. Clin. Lab. Invest. *37*, 357–361.
32. Borup, P., Kordac, V., Pedersen, J. S. & With, T. K. (1980) The porphyrin pattern in normal urine. Int. J. Biochem. *12*, 1075–1080.
33. Rocchi, E., Gilbertini, P., Santunione, V., Balli, F. & Ventura, E. (1980) Faecal and urinary coproporphyrin isomers in biliary atresia and neonatal hepatitis. Ricerca Clin. Lab. *10*, 501–509.
34. Kondo, T., Kuchiba, K. & Shimizu, Y. (1976) Coproporphyrin isomers in Dubin-Johnson syndrome. Gastroenterology *70*, 1117–1120.
35. Doss, M., Meinhof, W., Look, D., Henning, H., Nawrocki, P., Dölle, W., Strohmeyer, G. & Filippini, L. (1971) Porphyrins in liver and urine in acute intermittent and chronic hepatic porphyrias. S. Afr. J. Lab. Clin. Med. *17*, 50–54.
36. Nordmann, Y., Amram, D., Deybach, J. C., Phung, L. N. & Lesbros, D. (1990) Coexistent hereditary coproporphyria and congenital erythropoietic porphyria (Günther disease). J. Inher. Metab. Dis. *13*, 687–691.
37. Watson, C. J., Perman, V., Spurrel, F. A., Hoyt, H. H. & Schwartz, S. (1959) Some studies of the comparative biology of human and bovine erythropoietic porphyria. Arch. Intern. Med. *103*, 436–444.
38. Tonz, O., Vogt, J., Filippini, L., Simmler, F., Wachsmuth, D. & Winterhalter, K. H. (1975) Schwere Lichtdermatose nach Phototherapie bei einem Neugeborenen mit kongenitaler erythropoetischer Uroporphyrie. Helvetica Paediatria *30*, 47–56.
39. Nicholson, D. C., Cowger, M. L., Kalivas, J., Thompson, R. P. H. & Gray, C. H. (1973) Isotopic studies of the erythropoietic and hepatic components of congenital porphyria and erythropoietic porphyria. Clin. Sci. *44*, 135–150.
40. Vannotti, A. (1954) *Porphyrins – Their Biological and Chemical Importance*; Hilger and Watts, London.
41. Kaiser, I. H. (1980) Brown amniotic fluid in congenital erythropoietic porphyria. Obstetrics & Gynecology *56*, 383–384.
42. Tsai, S. F., Bishop, D. F. & Desnick, R. J. (1988) Human URO-III-S: molecular cloning, nucleotide sequence and expression of full-length cDNA. Proc. Natl. Acad. Sci. USA *85*, 7049.
43. Deybach, J. C., de Verneuil, H., Boulechfar, S., Grandchamp, B. & Nordmann, Y. (1990) Point mutation in the uroporphyrinogen III synthase gene in congenital erythropoietic porphyria. Blood *75*, 1763–1765.
44. Warner, C. A., Yoo, H. W., Roberts, A. & Desnick, R. J. (1992) Congenital erythropoietic porphyria: identification and expression of exon in mutations in the uroporphyrinogen III synthase gene. J. Clin. Invest. *89*, 693–700.
45. Boulechfar, S., Da Silva, V., Deybach, J. C., Nordmann, Y., Grandchamp, B. & de Verneuil, H. (1992) Heterogeneity of mutations in the uroporphyrinogen III synthase gene in congenital erythropoietic porphyria. Human. Genet. *88*, 320–324.

Pr. A. Hassoun
Service de Biochimie Médicale
Cliniques Universitaires St Luc
10 avenue Hippocrate
B-1200 Bruxelles
Belgique